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Screening environmental samples for source-specific bacteriophage hosts using a method for the simultaneous pouring of 12 petri plates

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A simple apparatus was developed to allow 12 petri plates to be poured simultaneously by hand. It was used when screening bacterial isolates from sewage and dog feces for their ability to detect phages from these sources. This was done to assess the ease with which source-specific phage hosts can be isolated from these sources of fecal pollution. Host bacteria that consistently detected phages from sewage were easily isolated from sewage. These bacterial isolates did not detect phages from dog feces. Host bacteria were not isolated from dog feces even after screening hundreds of colonies from fecal samples from six dogs. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 124–126.

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Introduction

Microorganisms such as fecal coliforms and coliphages are used as indicators of fecal or sewage pollution in water [5]. Although these indicators reveal the presence of fecal pollution, they do not differentiate sources of fecal pollution. Interest in source identification emerges when attempts are made to eliminate fecal pollution into bodies of water. Somatic coliphages are a diverse group of phages found in water systems subject to pollution with fecal wastes [2,3]. Different sources of phages such as sewage or dog feces probably have dissimilar phage-host assemblages. It may be possible to isolate bacteria, unique to a given source, that are host to specific phages from that source. These source-specific hosts could then be used as indicators of phages from that source.

We made an apparatus with which we could pour 12 petri dishes simultaneously. This allowed us to screen bacterial isolates from sewage and dog feces rapidly for their ability to detect specific phages from these sources. Our aim was to determine the ease with which source-specific phage hosts could be isolated.

Materials and methods

Screening for host bacteria from a specific source

Bacterial isolates were grown on tryptone-glucose-yeast extract (TGYE) agar or in TGYE broth. Coliphage ϕX174 and its host, *Escherichia coli* C, were used as positive controls.

Sewage influent was collected from a sewage treatment facility at Boston Harbor, MA and brought to the lab on ice. Samples of dog feces were collected from dogs kept in private homes and from dogs kept in kennels. Portions of dog feces were mixed in TGYE broth, centrifuged ($6000 \times g$ for 10 min at 4°C) and the supernatant was used for isolation of phages and host bacteria.

Diluted samples were spread on TGYE agar and incubated overnight at 37°C. Isolated colonies from these plates were picked and inoculated in TGYE broth. After overnight incubation at 37°C, 1 ml of each bacterial isolate was mixed with 4 ml of soft agar and 0.1 ml of sewage or dog fecal supernatant. This was then poured over TGYE (bottom) agar according to double-agar overlay procedures [1]. Double-agar overlay plates were incubated at 37°C and examined the next day for plaques. Bacterial isolates that had plaques on their lawns were saved and additional sewage and fecal samples were tested on these host bacteria.

Pouring multiple plates

Figure 1 shows a diagram of an apparatus that holds an array of 12 plates in a stepwise fashion. This allows 12 tubes of soft agar, kept fairly close together in a suitable holder, to be poured simultaneously into the plates. Several tube racks, each with 12 tubes of agar in a single row, were placed in a perforated aluminum rack-holder and autoclaved to melt the agar. The rack-holder along with its racks supporting tubes of melted agar was then placed in a water bath set at 50°C. Samples and host bacteria were added to the 12 tubes using manifolds made to hold disposable micropipette tips spaced so each tip corresponded to one tube. The rack of 12 tubes was then shaken to effect mixing and the tubes poured so each plate received the contents of one tube.

Results and discussion

Sixteen of 108 bacterial isolates from sewage consistently detected phages in sewage when tested on three sewage samples collected on different weeks. These 16 sewage hosts were tested on three more sewage samples (Table 1)

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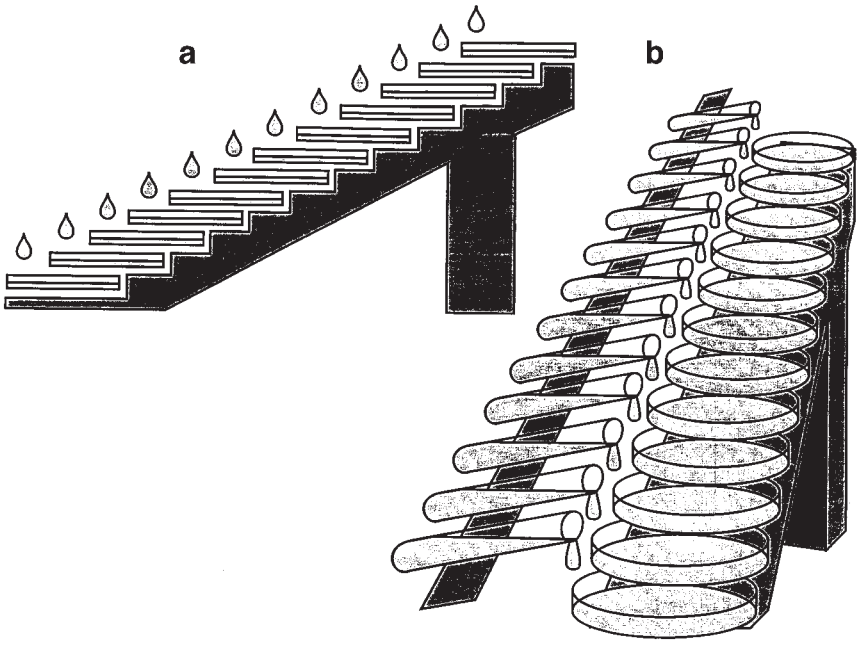

Figure 1 Side view (a) and perspective (b) of a step-like holder for petri plates used to pour 12 plates at once. Test tubes containing molten agar are held in a single row, spaced to allow the simultaneous pouring of each into a corresponding plate.

Table 1 Plaque counts (pfu per 0.1 ml) from sewage tested on 16 bacterial isolates from sewage

Isolate number	Sewage sampled on		
	19 October	28 October	6 November
1	0	1–10	6
2	85	1–10	11
3	1–10	1–10	12
4	10–50	1000	42
5	22	28	13
6	10–50	44	13
7	90	119	118
8	1–10	16	4
9	10–50	1–10	92
10	0	0	10
11	0	0	2
12	10–50	17	4
13	10–50	1–10	24
14	93	41	48
15	72	66	8
16	0	1–10	19
E. coli C	183	79	164

and consistently detected phages in sewage. Isolates No. 4, No. 7 and No. 14 detected the highest phage numbers. Plaque counts on isolate No. 7 approached plaque counts obtained using *E. coli* C as host. *E. coli* C is a lab strain which has a variety of surface receptors sensitive to numerous coliphages and lacks a restriction system [5]. Therefore it is suprising to find an environmental isolate with a comparable sensitivity to phages in sewage.

None of 504 bacterial isolates from six fecal samples from dogs detected phages in dog feces. Two isolates from one fecal sample exhibited plaques when fecal matter from the same sample was tested on them but these isolates did not show any plaques when subsequent fecal samples were tested. Only one fecal sample had somatic coliphages when tested on *E. coli* C. This sample showed hundreds of tiny plaques. The other samples tested for somatic coliphages showed none. All of these dogs were from private homes. Their isolation from other dogs and clean living conditions may have contributed to the low phage numbers in their feces. Somatic coliphages are sometimes, but not always, present in dog feces [4,6]. On another occasion, we tested 16 fecal samples from dogs in a kennel for somatic coliphages. Somatic coliphage counts varied from none to greater than 10^7 pfu g^{-1}. These dogs lived in conditions where recontamination by microbes in their feces was more likely.

Making a stand to hold 12 plates in a stepwise configuration was not difficult. Neither was it hard to make tube holders, pipette tip manifolds or other supporting apparatus. The ability to pour 12 plates simultaneously reduced the time spent pouring plates by ten-fold. It was not hard to line up the tubes with each plate or to pour the contents of each tube into a plate. This method should be useful in any experiment involving pouring individual samples into large numbers of plates.

For our double-agar overlays, the quantity of agar poured for the top layer was too small to spread completely over the bottom agar. This was rectified by picking up the whole step-apparatus and swirling the agar in the plates at the same time in order to get the top agar to spread evenly over the entire surface of the bottom layer.

In summary, it was easy to isolate bacteria from sewage that detected phages in sewage consistently. In spite of screening hundreds of bacterial isolates from six fecal samples from dogs, no isolate was suitable for detecting phages from dog feces. Low numbers of somatic coliphages in the feces of these dogs suggests that phage-host systems

were not there. The multiple-plate pouring method was useful for screening many bacteria for their ability to detect phages. This method was easy and reliable and greatly reduced the time spent pouring plates.

Acknowledgements

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